2194-001-0
80/

TITLE OF THE INVENTION

ELEMENT AND METHOD FOR PERFORMING BIOLOGICAL ASSAYS ACCURATELY, RAPIDLY AND SIMPLY

BACKGROUND OF THE INVENTION

Field of the Invention:

The present invention relates to disposable reagent-containing elements which can be used in conjunction with an electronic instrument for performing diagnostic assays (medical diagnostics). It also relates to a method for performing diagnostic biological assays employing the use of a disposable reagent-containing element.

Discussion of the Background:

Many analytical techniques have been developed for chemical, biochemical and biological assays. Procedures that use a discrete fluid sample for the analysis of a single analyte are traditionally characterized as wet chemical techniques or dry chemical techniques. In recent years both types of techniques have been automated to reduce costs and simplify procedures. Wet chemical methods, typified by the Technicon® autoanalyzers, utilize batches of reagent solutions, pumps and fluid controls, coupled with

conventional sensors such as radiometric (e.g.: fluorescent, colorimetric, or nephelometric) electrochemical (e.g.: conductometric, polarographic, or potentiometric) and others, such as ultrasonic, etc. sensors. These techniques are characterized by large equipment, and are generally expensive. They are complicated, and require a skilled operator.

Decentralized testing, particularly in medical applications, has been achieved with a variety of simpler systems often based on cuvettes for optical determination but sometimes based on dry chemistry-based "reagent strip" technology. Generally, a reagent strip is an absorbent structure containing a reagent which self-meters an applied sample and develops or changes color to indicate the extent of reaction. Although self metering, some reagent strips, however, require pipetting of sample to achieve maximum accuracy and precision. A reagent strip is employed either by itself or in conjunction with a simple instrument to read the color intensity or hue and translate the results into a numerical value which is displayed. Unlike a cuvette or test tube, mixing or convection cannot be sustained in a reagent strip after the sample has entered, and once having entered, the sample cannot be removed from within without destroying the integrity of the strip. In one application, reagent strip

technology is used extensively in the home by diabetic patients who test themselves daily to determine blood sugar levels.

In a more general example of "reagent strip" technology, Eastman Kodak has introduced a system of dry chemistry, claiming to overcome many of the traditional weaknesses of dry chemical methods. The Kodak technique utilizes flat, multi-layered sheets arranged in sequence. The top layer receives a liquid sample which passes downward undergoing separation and reactions in a pre-arranged sequence. The sheet is designed to accept a small volume of liquid and distribute it uniformly over a reproducible area. The area is less than the total area of the multi-laminar sheet. Each layer of the sheet is essentially homogenous in a direction parallel to the surface. Once the sample has spread radially (a rapid process), the components of the liquid can move downward at rates that are essentially the same in any plane that is parallel to the surface. In this way uniform reactions, filtrations, etc. can occur.

The analyte is detected in the multi-layered sheets by radiometric or electrochemical methods which are carried out in a thermostated environment. This permits the use of kinetic and static measurements to detect analyte concentrations in a liquid sample.

Radiation is caused to enter this assembly in a path which is traverse to the several layers. The radiation is modified by the analyte or by a component or product of the analyte. For example, the exciting radiation may be partially absorbed by the analyte or by a component or product of the analyte. The modified radiation may be reflected back transversely through the laminar assembly, typically from a reflective layer, adjacent or nearly adjacent to a thin layer where color is formed. Thus, reflectance can be monitored (as opposed to transmission only through the color producing layer). Reflectance, as expressed by Kubelka-Monk theory, consists of optical density absorbance and scattering components and is more sensitive than transverse colorimetry through a thin, turbid layer. Reflectance, however, may be a more difficult technique to standardize and to interpret data from than colorimetry.

Conventional colorimetry has not been practiced with reagent strips because the color producing layers are generally thin and not transparent. The path of the exciting radiation is thus very short (with large light losses due to opacity) and is determined by the thickness of the layer in which the exciting radiation encounters the substance which is excited. Since this dimension must be very small to permit rapid

measurement, e.g., 10 μm to 100 μm, the degree of modification of the exciting radiation is quite small. This limits the applicability of this technique to analyses in which the analyte (or the product of the analyte) interacts very strongly with the exciting radiation, otherwise a very sensitive detecting apparatus has to be used. This method has been shown to be useful for measuring analytes in blood that exist at relatively high concentrations, e.g., glucose, BUN, cholesterol and albumin.

Other analytical methods have been developed that utilize rapidly reversable chemical reactions to continuously monitor analyte concentrations in biological fluids, or industrial effluent streams, or ponds, lakes and streams. For example, several methods have been proposed to measure the oxygen level in blood of critically ill patients.

Reagent strip technology, however, possesses salient drawbacks and limitations. For example, once a sample is added to a reagent strip and permeates the porous structure of the strip, the sample cannot be removed or washed out without destroying the integrity of the strip. For example, immunoassays are extremely difficult to perform with reagent strips, in part because separation of free and bound antigen (or antibody) molecules from a mixture of both cannot be

readily achieved in a conventional porous or layered structure. This limits possible immunoassay applications to certain special cases of reactions, for example certain homogenous reaction sequences. Incubation with mixing, a step common to a variety of assays, cannot be performed easily in conventional reagent strip formats since they rely on diffusion and initial capillary action only for mixing.

Technologies have not yet been developed to cause or to control forced convection for a specified period of time within the porous structure of a reagent strip after the sample has entered and permeated the porous strip structure. In conventional reagent strips, the strip is an absorbant matrix in which mixing is extremely difficult and limited. In addition, reagent strips almost exclusively use reflectance as a photometric method to quantitatively determine the extent of a color reaction. There are no reagent strips known to the inventor which can be read via light transmission/absorbance colorimetry, nephelometry, fluorescence, chemiluminescence, or evanescent wave technology. Fluorescence measurement is possible in reagent strips, but difficult to achieve. Electrochemistry has been used successfully.

As discussed above, a large number of types of medical tests are carried out by trained medical

laboratory personnel. These tests must be performed accurately and reproducibly with a minimum amount of error since they are used as aids in diagnosing and treating medical ailments. To aid laboratory personnel in performing these tests accurately on a large number of samples in a relatively short period of time, auxiliary equipment, which is often expensive, is frequently used. Most of these tests are performed on a macro scale and thus require considerable quantities of both sample and reactants. They also require varying degrees of sample preparation. These and other reasons are major contributors to the generally relatively expensive nature and inaccuracy of medical diagnostic tests performed on body fluids.

Improvements have been made in some medical tests. For example, the reagent strip technology discussed previously simplifies medical tests, minimizes the required quantities of sample and/or reactants, can minimize possible sources of error, and lower costs. Various types of medical tests, however, have been difficult to perform accurately and economically on either a macro or a micro scale. In this respect, medical tests which require rapid and thorough mixing of reagents with a sample to provide a clearly defined starting point, an accurate measurement of reaction time, and a clear determination of the

reaction endpoint, have been particularly difficult to perform with simple and inexpensive devices and have been plagued with inaccuracies resulting from errors in measurement and manipulation.

Once such type of test is the blood prothrombin time test ("PT" hereinafter). This test measures the time required to form a blood clot (via extrinsic and common blood coagulation physiologic pathways).

Coagulation assays, in general, are used for a variety of reasons. They are principally used for monitoring patients receiving anticoagulant therapy. The most frequently performed coagulation assay is PT. Prothrombin time determinations are used to monitor patients receiving oral anticoagulants such as warfarin. An accurate monitoring of coagulation in these patients is important to prevent recurrent clotting (thrombosis) and to keep the coagulation mechanism sufficiently active to prevent internal bleeding. Prothrombin time testing provides information to permit better drug control to be achieved through the regulation of drug dosage.

In conventional practice, PT assays are performed by the addition of a liquid reagent to a plasma sample. The reagents are typically supplied in dried form and consist primarily of thromboplastin and calcium chloride. The dried reagent is reconstituted

before use by addition of a measured amount of distilled water. The reagent is thermally sensitive, and refrigeration prior to use is required. The shelf life of the reagent in dried form is from one to two years. However, when it is reconstituted the reagent is considerably more labile and must be used within a few hours or discarded. In some cases reconsistuted reagents can be kept for a few days under refrigeration.

Prothrombin time assays are performed by mixing sample and reagent at 37°C, and monitoring the progress of the reaction until a perceptible clot (or "gel clot") is detected. The development of a gel clot is the end point of the reaction. This end point may be detected in various ways; by viscosity change, by electrode reaction; and, most commonly, by photometric means. The test result is generally compared to a result using a normal (control) plasma.

Before performing the test, the blood sample is collected in the tube or syringe containing anti-coagulant (citrate). The blood sample is centrifuged, and the plasma separated (e.g., by decantation) from the red blood cells. A measured quantity (usually 0.1 ml) of plasma is pipetted into the reaction vessel or cuvet. A measured amount of reagent is then added manually via pipette or automatically by means of other

volumetric delivery systems capable of metering a known, preset quantity of reagent. Alternatively, the sample can be added to the reagent directly. Typically, 0.2 ml of reagent is employed. The addition of the reagent initiates the reaction.

Some PT kits for use in the home are known. For example, McCormick (United States Patent No. 3,233,975) discloses a prothrombin reaction chamber. The chamber is constructed of a transparent material so that the progress of the reaction can be visually monitored. To perform a blood prothrombin time test with this chamber, one adds sequentially a measured volume of a prepared blood sample and a measured volume of an aqueous solution of reagent to the chamber. The chamber is then manually agitated, and the progress of the reaction visually monitored and timed with a stop watch.

This prothrombin reaction chamber, however, suffers from numerous disadvantages. For the prothrombin test to be performed with this reaction chamber, a prepared blood sample is used. Thus sample manipulation is required. A specific volume of the prepared blood sample must be added to the chamber. The measurements involved in obtaining this specific volume of prepared blood sample contribute inaccurate results and considerable labor.

This reaction chamber also requires the preparation of a solution containing the reagent(s). The precise measurement of the amounts of materials and water to be combined in preparing the reagent solution introduces another additional source of error. The measurement of the quantity of reagent solution to be added to the chamber provides a further source of error. Moreover, as discussed above, having to use a reagent solution is undesirable because of potential stability problems. If the reagent solution is not used within a few hours, the solution must be discarded.

McCormick's prothrombin reaction chamber is based on the visual observation of the reaction to measure clotting time. It does not permit accurate monitoring of sample mixing with the reagent(s), accurate determination of reaction starting point (which is as important as the end point when reaction time is being measured), or accurate determination of reaction end point.

Accordingly there is a strongly felt need for a facile and accurate method for the performance of biological assays, e.g., in medical application. Such method should be based on a minimum number of manipulations of either a sample or reagent.

Ideally it should require no sample or reagent-containing solution preparation. It should minimize problems associated with reagent instability and optimize accuracy. It should permit effective mixing of sample and reagent. It should permit sample manipulation. It should require only a very small amount of sample. And it should be able to perform automatic treatments of the sample, e.g., separate red blood cells from plasma in blood. This method should be based on a simple and inexpensive reagent-containing element.

SUMMARY OF THE INVENTION

Accordingly, it is an object of this invention to provide a facile method for the performance of assays, e.g., biological assays.

It is another object of this invention to provide a facile and accurate method for the performance of assays, e.g., biological assays.

It is another object of this invention to provide a method for the performance of assay where the method is based on a minimum number of manipulations of either sample or reagent.

It is another object of this invention to provide a method for the performance of assays requiring no preparation of sample or reagent-containing solution.

It is another object of this invention to provide a method for the performance of assays which minimizes problems associated with reagent instability.

It is another object of this invention to provide a method for the performance of assays in which accuracy is optimized.

It is another object of this invention to provide a method for the performance of assays permitting an effective mixing of sample and reagent(s).

It is another object of this invention to provide a method for the performance of assays permitting sample manipulation.

It is another object of this invention to provide a method for the performance of assays requiring only a very small amount of sample.

It is another object of this invention to provide a method for the performance of assays permitting the automatic treatment of the sample, e.g., separation of the red blood cells from plasma and blood.

It is an object of this invention to provide a novel element which permits the facile and accurate performance of diagnostic assays.

It is another object of this invention to provide such an element which can be used in diagnostic assays without requiring the preparation of a reagent solution from dry reagent.

It is another object of this invention to provide such an element permitting the accurate testing of samples with minimum sample manipulation.

It is another object of this invention to provide a novel element for performing a diagnostic assay in which no measurement of sample or reagent is required for performance of the assay.

It is another object of this invention to provide a novel element for performing a diagnostic assay permitting an optimization of accuracy.

It is another object of this invention to provide a novel element for performing a diagnostic assay permitting the effective mixing of sample and reagent(s).

It is another object of this invention to provide a novel element for performing a diagnostic assay permitting sample manipulation.

It is another object of this invention to provide a novel element for performing a diagnostic assay requiring only a very small amount of sample.

It is another object of this invention to provide a novel element for performing a diagnostic assay capable of automatically treating the sample, e.g., separating red blood cells from plasma and blood.

Surprisingly, all of these objects, and other objects which will become obvious from a description of

the invention provided hereinafter, have all been satisfied with the discovery of the present reagent-containing element for performing diagnostic biological assays. This element comprises a channel structure defining a sample well and a reaction volume in communication with each other. The channel structure possesses a geometry which causes a liquid sample placed into the sample well to be drawn into and filling the reaction volume via capillary action, wherein after the reaction volume is filled, the liquid sample remains stationary.

In the assembly of this element, the element can comprise a base, an overlay, and a cover. The base comprises a major surface. The overlay is situated on the base. The cover is situated on the overlay, opposite the base. The overlay comprises a channel structure defining a sample well and a reaction space in communication with each other. The cover comprises a means for adding a sample to be analyzed to the sample well.

The assay is performed by monitoring a reaction of the sample in the reaction space with the sample as a whole being stationary during essentially all of the assay. After the assay, or to permit manipulation of the sample, the sample can be removed from the reaction volume, but the geometry of the present element provides for the immobility of the sample once the reaction volume has been filed.

Although the element can comprise the base, overlay and cover assembly described above, it can be produced by using any material forming technique which will produce the desired geometry. Thus, instead of assembling the base, overlay and cover as separate components, the element can be produced by assembling either fewer or a greater number of components. For example, the element can be produced by injection molding whereby one obtains two pieces which when assembled produce the element of this invention.

In certain embodiments, the element also comprises a means for channelling light from an outside source to the reaction chamber. This means is referred to as a waveguide in this text. Such an element is used with a means for detecting light emitted from the reaction chamber. Other embodiments measure non-optical properties of the sample. Such measurements may, for example, be conductometric, polarographic or potentiometric in nature, or may involve a combination of the above.

Of course, the present invention also provides a novel method for performing a diagnostic assay. This method is based on using the reagent-containing element of this invention. The assays which can be performed are all liquid system assays, i.e., assays using a liquid media. A specific example of such assays is biological assays.

An element containing a measured amount of at least one reagent situated in the reaction space of the element, is used. A biological sample is added to the sample well of the reagent-containing element. The geometry of the reagent-containing element forces a specific volume of the biological sample to be drawn from the sample well by capillary action to the reaction space. In the simplest case of reagent containment, once the sample enters the reaction space it contacts the reagent, dissolving the reagent. Using the means for channelling light from an outside source to the reaction chamber, light is impinged upon the reaction space during the whole process. Light emitted from the reaction space is monitored, permitting monitoring of the dissolution process, the progress of the reaction, the end of the reaction, and a determination of reaction time.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same becomes better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein like reference numerals designate identical or corresponding parts throughout the several views.

FIGURE 1 is a top view of a cover of a first embodiment of a reaction slide according to the current invention.

FIGURE 2 is a top view of an overlay of a reaction slide according to the first embodiment.

FIGURE 3 is a top view of a base of a reaction slide according to the first embodiment.

FIGURE 4 is an exploded perspective of the items shown in FIGURES 1-3, the elements being oriented as in the assembled reaction slide.

FIGURE 5 is a top view of the elements of FIGURES 1-3, when assembled.

FIGURE 6 is an elevational longitudinal cross-section of a first embodiment of a reaction slide according to the current invention, the cover, overlay and base being sectioned along line VI-VI of FIGURE 5.

FIGURE 7 is an elevational cross-section of a fragment of a reaction slide according to the current invention, illustrating a modification in which the reaction slide comprises a spacer that includes two overlays.

FIGURE 8 is a top view of a second embodiment of a reaction slide according to the current invention.

FIGURE 9 is a top view of a cover of a third embodiment of a reaction slide according to the current invention.

FIGURE 10 is a top view of an overlay of the third embodiment.

FIGURE 11 is a top view of the base of the third embodiment.

FIGURE 12 is a top view of the third embodiment.

FIGURE 13 is an elevational cross-section taken along line XIII-XIII of FIGURE 12, further showing a liquid absorbing matrix.

FIGURE 14 is a top view of a fourth embodiment of a reaction slide according to the current invention.

FIGURE 15 is an exploded view of a fifth embodiment of a reaction slide according to the current invention.

FIGURE 16 is a top view of the fifth embodiment.

FIGURES 17 and 18, respectively, are elevational cross-sections taken on lines XVII-XVII and XVIII-XVIII of FIGURE 16.

FIGURE 19 shows a transverse cross-sectional elevation of a reaction slide, a preferred embodiment of a light source, also in section, and a light detector, the light source and light detector being disposed for making a reflectance measurement.

FIGURE 20 is a top view of a reaction slide disposed in a housing for making a reflectance measurement, a cover of the housing being removed.

FIGURE 21 is an elevational cross-section taken on line XXI-XXI of FIGURE 20, also showing the cover of the housing.

FIGURE 22 is an exemplary graph showing typical results of a measurement of prothrombin time.

FIGURE 23 schematically illustrates apparatus for measuring prothrombin time.

FIGURE 24 schematically illustrates a vertical cross-section of a modification of a reaction slide that does not employ adhesive layers, the figure illustrating light entering that embodiment.

FIGURE 25 illustrates the use of a reaction slide (unsectioned), external waveguides and apparatus for making a transmission/absorbance measurement.

FIGURE 26 is a view similar to that of FIGURE 25, illustrating simultaneous measurements of light scattering and transmission/absorbance.

FIGURE 27 illustrates a reaction slide and a light detector, disposed for making a measurement based upon chemiluminescence.

FIGURE 28 shows a reaction slide disposed above a partial integrating sphere for making a measurement based on reflectance.

FIGURE 29 illustrates simultaneous measurements based on light scattering and transmission/absorbance through the reaction space and the use of the cover in making a fluorescent evanescent wave measurement.

FIGURE 30 illustrates the use of the base of a reaction slide in making a fluorescent evanescent wave measurement.

FIGURE 31 illustrates the use of a screen for setting up convective currents in the reaction space.

FIGURE 32 illustrates the use of a permanent magnet for setting up convective currents in the reaction space.

FIGURE 33 illustrates the use of a solenoid for setting up convective currents within the reaction space.

FIGURE 34 illustrates apparatus for producing localized deflection of the cover to produce convective currents within the reaction space.

FIGURE 35 is a transverse elevational cross-section of a reaction slide provided with a calorimetric transducer.

FIGURE 36 is a top view of a reaction slide, with the cover removed, the reaction slide being provided with an electrochemical transducer.

FIGURE 37 is a transverse cross-sectional elevation of a reaction slide provided with a viscosity transducer.

FIGURE 38 is a longitudinal cross-sectional elevation of a reaction slide augmented for performing a continuous flow measurement and having a reagent-containing layer disposed on the base.

FIGURE 39 is a fragment of FIGURE 38 in the area of the reaction space, in which the reagent-containing layer is in the form of a reagent-containing gel.

FIGURE 40 is a fragment of FIGURE 38 in the area of the reaction space in which the reagent-containing layer is in the form of a reagent-containing membrane disposed above a liquid absorbing matrix.

FIGURE 41 is a fragment of FIGURE 38, modified by the addition of a recess in the base to accomodate a liquid absorbing matrix and a second reagent-containing layer.

FIGURE 42 illustrates a reaction slide modified for use in initiating an assay.

FIGURES 43-51 are longitudinal cross-sectional elevations of a reaction slide during various stages of an ELISA type immunoassay.

FIGURES 52-60 schematically illustrate the physiochemical conditions within the reaction space during each of the stages illustrated in FIGURES 43-51.

FIGURE 61 is a longitudinal cross-sectional elevation of a reaction slide in which a reagent-containing matrix fills a substantial portion of the reaction space.

FIGURE 62 is a top view, with the cover partially removed, of independent reaction spaces disposed on a common base.

stiffness led to cracking and leakage during repeated mechanically-induced mixing.

d) A polyethylene terephthalate sheet coated on both sides with acrylic adhesive (total thickness of 0.004 inches) worked well as a spacer and waveguides and provided the necessary flexibility and leak resistance during filling and during mechanical mixing operations.

e) A spacer made from acrylic adhesive only (approximate thickness 0.002 inches) provided flexibility, but contained many inhomogeneities, scattering a considerable amount of light and providing a marginally acceptable waveguide.

f) A reaction slide was fabricated as in Example 1 but employed a 0.050 inch thick, 25 x 75 mm precleaned glass microscope slide as a base material. In assay performance this material worked extremely well and was comparable to the polycarbonate base.

g) A reaction slide prepared as in Example 1 but with a 0.007 inch glass cover, carefully cut to shape, worked well in short term studies for light scatter measurement but was prone to cracking during long term mixing studies.

h) Reaction slides prepared as in Example 1 with reaction volumes of the same dimensions, but with extended cover, spacer, and base (to 2 inches in width)

provided attenuated transmitted light from the source into the reaction volume. A low density polyethylene (LDPE) overlay 0.007 inches thick when secured with acrylic adhesive to the cover and base provided a signal which was too weak to be easily distinguished from noise, and therefore this slide was not usable at this distance. The same film was, however, acceptable as a waveguide over a smaller distance (0.125 inches). A polyvinyl chloride overlay (which scattered light less and provided a better waveguide material) was successfully employed in this experiment in place of the LDPE in both instances (at 0.125 and at 2 inches).

i) A reaction slide was prepared as in Example 1 with 0.1% Triton X-100 surfactant added to the reagent. Results comparable to Example 1 were obtained, and the reaction slide reaction volume was easier to fill. Prewashing cover and base with the same concentration of surfactant produced similar results.

Example 7

A reaction slide prepared as in Example 1 was utilized in a similar experiment with the same plasma sample. In this case, sustained mixing was achieved by means of a 3-inch long push rod glued to and driven by

a 3-inch diameter 8-ohm electromagnetic speaker coil. The cylindrical push rod tip diameter was 0.1 inch and pushed against the cover producing oscillations of the cover. The downward deflection distance was approximately 0.005 inches under an applied force of approximately 3 ounces. A 9-volt square wave driving signal was used with 0.2 second duration pulses every second. The resulting higher frequency cover deflection induced light intensity fluctuations that were superimposed as tiny ripples on the relatively lower frequency scatter curve and could be observed as a tiny ripple without obscuring the signal. The endpoint, however, appeared sooner than that observed in Example 1 and was occasionally sharper.

Example 8 - Plasminogen Activator Assay

A reaction slide was prepared as in Example 1 but filled with a mixture for measuring quantitatively the concentration of plasminogen activator in a sample. The assay method of Campbell was adapted accordingly: E.E. Campbell, et al. (Clinical Chemistry 28, No. 5, 1982, pp. 1125-1128). The mixture consisted of 25 parts (by volume) 0.33 mg/ml plasminogen in 0.1 M sodium phosphate buffer; 20 parts S-2251 (D-Val-Leu-Lys- p nitroanilide) 75 mM in deionized water; and 10 parts fibrin monomer 3.3 mg/ml in phosphate buffer/urea

(0.02 M sodium phosphate, 0.3 M sodium chloride, and 3 M urea). After filling, the reaction slide was freeze-dried. After freeze-drying the reagents in the reaction volume, the slide was tested as follows: A sample of plasminogen activator was added to the sample well of a reaction slide prewarmed to 37°C. The sample contained 1000 units of activator in TRIS buffer (50 mM Tris, 150 mM sodium chloride, pH 7.4). After a few minutes, a bright yellow color appeared, indicating the presence of activator. This visually apparent color could easily be seen or readily monitored by placing the slide against a white reflective background (reflectance measurement). Alternatively, the color could be read colorimetrically via absorbance.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of the appended claims, the invention may be practiced otherwise than as specifically described therein.